NUCLEIC ACIDS

Ribosomes Revived

from our Molecular Biology Correspondent

"THE recognition of specific nucleotide sequences by proteins represents one of the more fundamental molecular processes in the biosphere." Teilhard de Chardin calling from the great beyond? By no means: it is Schaup, Green and Kurland who get their paper (*Mol. Gen. Genet.*, **109**, 193; 1970) off to a flying start with this intoxicating declaration. They then go on to describe how some of the proteins of the 30S ribosome of *Escherichia coli* will under defined circumstances bind only to their allotted sites on the 16S RNA, and they imply that, because RNA sequencing is now a routine skill, the auguries for determining the sequence of specific protein binding sites on the RNA are excellent.

It is known from the work of Nomura's group that the primary step in reassembly of the 30S particle involves the binding of only a handful of "core" proteins to the RNA, and this is borne out by the work of Schaup *et al.* who find that only five out of the sixteen 30S proteins that they tried were able to form defined complexes with the 16S RNA in a medium of high salt concentration containing 0.02 M magnesium. The conditions are important, for at lower concentrations the association is obscured by the kind of non-specific binding expected between RNA and any moderately basic protein. The combining ratios of RNA and each individual protein can be determined by incremental addition of labelled protein, and measurement of the amount of protein associated with the RNA after separation from unbound protein by sucrose gradient centrifugation, gel filtration or electrophoresis. Of the five proteins displaying strong binding, four show unquestionable saturation at 1 : 1 and one has undefined stoichiometry. To show that each protein enters a unique site, competition experiments were performed, in which the binding of a ^{14}C-labelled protein was measured in the presence of tritium-labelled proteins. There was no competition except when the additional protein was the same species as the binding protein. Instead, however, there was clear evidence of enhancement of binding of some proteins by others. This cooperative element was to be anticipated from Nomura's results. Some discrepancies between his classification of the proteins into binding and non-binding categories and those of Schaup *et al.* are put down to conformational or oxidative damage on standing. There is also an allusion to evidence of interaction of two 30S proteins with the RNA from the 50S particle, and this may relate to the finding of Nomura's group that the reassembly of the 50S particle is promoted by the presence of intact 30S subunits.

A prominent feature of the reconstitution process is that it requires transient exposure to a higher temperature. This is a condition for correct refolding of an unfolded or incorrectly folded intermediate. That more subtle manifestations are also involved is now shown by Miskin, Zamir and Elson (*J. Mol. Biol.*, **54**, 355; 1970), who have examined the requirements for peptidyl transferase activity in the 50S ribosome. This is assayed by Monro's procedure whereby, in the presence of high concentrations of alcohol, formylmethionine is transferred from fMet-tRNA to puromycin. The reaction, it turns out, is characterized by an absolute requirement for monovalent cations, of which ammonium and rubidium are the most effective, potassium nearly so, and sodium and lithium do not work. Magnesium must also be present. The ribosome can be reactivated by adding the monovalent cations back, but this reactivation is vanishingly slow at low temperature, whereas it takes only a few minutes in the neighbourhood of 40°C. The reactivation is first order, but shows a bent Arrhenius plot, which may reflect conformational adjustments. In terms of sedimentation and optical characteristics the activation involves no gross change in structure, and may therefore be highly localized. The authors note that under the standard conditions of assay reactivation will occur, so that the assay must be conducted at low temperature in any search for an equilibrium between active and inactive states.

That such considerations may have a much wider relevance than has been envisaged hitherto is suggested by the accompanying paper from the same laboratory (Vogel *et al.*, *ibid.*, 379), which demonstrates the existence of a similar temperature-dependent transformation in 30S ribosomes, assayed in terms of poly U-stimulated phe-tRNA binding. More particularly, streptomycin will bind to the 30S ribosome only when it is in the active form. This binding is immeasurably fast, and the slow binding process observed by earlier workers is thus to be explained in terms of rate-determining reactivation at the temperature of the measurements. Three antibiotics that operate on the 50S particle follow the same pattern: binding occurs only when the ribosome is in its active state. What delicate structural disturbance might control the activation equilibrium it is scarcely useful to speculate, but these results might give a number of workers in the field cause for deep reflexion.

Floating to Synchrony

BIOCHEMISTS who are clamouring after synchronous cultures of mammalian cells, for without them it is extremely difficult if not impossible to define the changes in a cell's biochemistry during the normal cycle of cell division, should be heartened by a report in next Wednesday's *Nature New Biology* by S. Shall and A. J. McClelland (**229**, 1971). These workers claim that synchronously dividing populations of LS cells, a line of L cell mouse fibroblasts which grows in suspension, can be obtained by carefully layering an unsynchronized population of cells at the top of a column of complete culture medium, and, after leaving the column for 50 min at 37° C, by removing the top millilitre. The cells recovered in this millilitre layer, about 2.25 per cent of the initial load, prove to be in the premitotic (G2) or very early mitotic stage of their growth cycle. In short, many, if not all, LS cells at these stages in the cycle float in conditions in which cells at other stages sink. This method has the great attraction of remarkable simplicity and speed, especially by comparison with the other techniques currently used for synchronizing cell populations: for example, sucrose gradient centrifugation, manipulation of the concentration of serum in the cell's medium and the physical detachment of mitosing cells.

Shall and McClelland's method is in many respects similar to the so-called "staput" gradient procedure exploited by Miller, Phillips and their colleagues in Toronto (*J. Cell Physiol.*, **73**, 191; 1969). It apparently relies on natural gravity to separate cells on the basis of their size rather than their density, but whereas the Toronto group added bovine serum albumin to stabilize their gradients Shall and McClelland, after first experimenting with sucrose gradients, found that they could dispense with such additives. At least with LS cells separation can be achieved in the same medium in which the cells are routinely cultivated.

Any technique which minimizes the changes in the medium of eukaryotic cells is an advance because the fickle behaviour of such cells and their sensitivity to changes in the composition of their medium are notorious. Serotonin, for example, as Boucek and Alvarez report in the same issue of *Nature New Biology* (**229**, 1971), alters the behaviour of several lines of human and murine fibroblasts although it has no detectable effects on other types of cells. In the presence of serotonin, subcultures of these fibroblasts start multiplying some 6 h sooner than control cultures in a medium lacking serotonin. This substance apparently accelerates the attachment of fibroblasts to their culture vessels, but that is not the whole story, for serotonin also increases the viability of fibroblasts during tissue culture.